THE CORTEX OF THE BRAIN IN THE HUMAN EMBRYO DURING THE FOURTH MONTH WITH SPECIAL REFERENCE TO THE SO-CALLED "PAPILLÆ OF RETZIUS"

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WITH 6 FIGURES

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The observations reported in the following paper conclusively show that the cortical granulations, or papillæ of Retzius, caused by the fungiform arrangement of the cells of the pyramidal layer, and commonly found in human embryos between 11 and 14 cm. long, constitute an abnormal condition, which is produced either by intrauterine or postpartum maceration. It is pointed out that of two human brains of the same age one may have cortical papillæ while in the other they may be absent. Furthermore, it is shown in pig brains, where cortical papillæ are not normally present, that it is possible by experimental methods to produce a fungiform clumping of the cortical cells that exactly duplicates the condition seen in human brains.

Attention was first called by Retzius, 95, p. 17, to the fact that human brains, usually of the fourth month and more rarely of the fifth month, possess a fine granulated character perceptible through the smooth surface of the cortex, and in places where the thin superficial layer (Randschicht of His) had been torn off they appear correspondingly granulated or covered with rounded elevations. Microscopical examination of sections of these regions revealed the fact that the granulated character was due to an unequal growth of the pyramidal cell layer, which projected in rounded elevations, the spaces between which were filled in by the superficial or molecular layer, so that the surface of the brain remained smooth. Retzius considered the possibility of this granulation formation being a manifestation of some pathological process, such as is commonly associated with abortion, *e. g.*, syphilis. He was, however, more inclined to believe it a normal condition due to a transitory exuberant growth of the pyramidal cell layer, the surface irregularities caused thereby being

smoothed out later by the development of the adjacent layers. Shortly after this Hochstetter, 98, p. 5, briefly refers to the granulations of the pyramidal cell layer described by Retzius. He confirms their presence in poorly preserved brains, and designates this appearance as a decomposition phenomenon, without giving any further evidence.

Two years later His, 00, not having noticed the observations of Retzius, described independently the same peculiar granular or wart-like character of the pyramidal layer in embryo brains of the fourth month; and again in his last work His, 04, he describes at some length this appearance under the title "Die Retziusschen Wärzchen." One of his illustrations is reproduced in Fig. 1. Though in his discussion he admits that it is

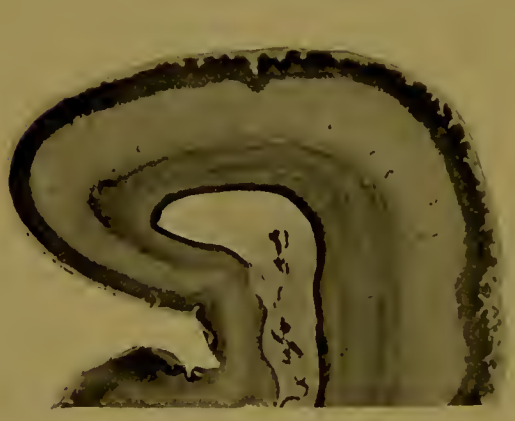

Fig. 1.

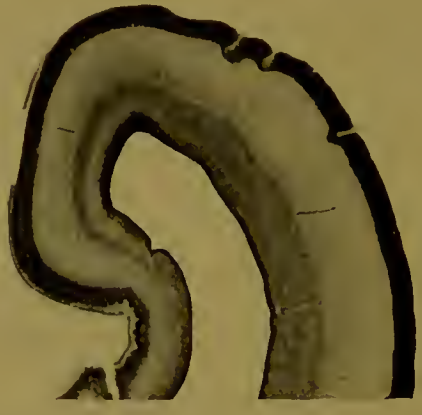

Fig. 2.

Fig. 1. Section through the occipital lobe of the brain of a human embryo, 12 cm. long (end of 4th month), showing the irregularities of the pyramidal zone caused by the fungiform clumping of the cortical cells, the so-called papillæ of Retzius. Taken from His, 04, Fig. 75.

Fig. 2. Section from the brain of a human embryo of about the same age and taken from the same place as shown in Fig. 1. Here the pyramidal zone consists of a compact layer with parallel borders, which, with the exception of three transitory fissures presents a perfectly smooth outer surface, and shows no trace of the Retzius papillæ.

still an open question; yet he is apparently inclined to consider the papillæ as normal, and does not hesitate to discard the possibility referred to by Retzius of their being pathological, on the ground that several of his specimens, which showed characteristic cortical papillæ, came from healthy individuals who had committed suicide, and the fœtuses themselves appeared normal. He also argues that if it were a post-mortem alteration, associated with the swelling of the tissues, then the superficial layer would also present an irregular surface, which is not the case.

Neither His nor Retzius accounted for the fact that this phenomenon

has not been observed in brains of other animals. With this in mind the writer decided to make the test on some other mammal, fresh embryos of which could be easily obtained at all ages, and where control experiments could be carried out on embryos of the same litter. The pig was selected, and an examination was made of brains of embryo pigs measuring from 10 to 14 cm. long, which is a period that corresponds to and fully covers the time of appearance of cortical papillæ in the human brain.[1]

It was found that the pyramidal layer in the pig does not exhibit any cortical papillæ when carefully preserved; but has always a smooth, regular surface, the only indentations being those corresponding to the beginning fissures, which make their appearance in specimens between 12 and 13 cm. long. A photograph of a section of a normal brain of a 11.5 cm. pig is shown in Fig. 3.

Having found that cortical papillæ are not normally present in the pig, the next step was to see whether they could not be produced artificially, and preferably under conditions which might be probable in case of human material. Two possibilities suggested themselves as etiological factors; in the first place, maceration of the specimen before it was put into the preserving fluid, and secondly imperfect penetration of the preserving fluid. Under maceration we should have to consider both postpartum and intrauterine maceration. The latter might be brought about, for instance, by disease of, or abnormal attachment of the placenta with consequent disturbance of circulation, and perhaps death of the fœtus some days before abortion. The second condition, faulty penetration of the fixative, might be present in brains of this size were the fixative not injected through the arteries or the brain coverings not immediately opened up so as to permit the direct action of the fluid on the brain itself.

The following experiments were carried out with the idea of imitating these two conditions; on the one hand, for obtaining imperfect penetration

[1] The fact that the cortical papillæ are usually limited to the fourth month may perhaps be explained as follows: Up to that time the brain wall is relatively thin and uniform in structure, so that deformities then take the form of complete foldings of the wall. In specimens of the fourth month the wall is sufficiently thick to prevent foldings of the entire wall, and expansion and shrinkage express themselves in a readjustment of its constituent parts, some parts being more affected than others. In older specimens such a readjustment is prevented by the development of the cell processes and the supporting framework of neuroglia, resulting in a structure sufficiently firm to preserve its form in the fixative, and consequently no more papillæ or artificial fissures are found.

of the fixing fluid, the brain coverings were not removed until the specimen was ready for embedding, and on the other hand, the maceration was produced either by keeping the specimens dry and exposed to the air long enough for them to macerate in their own fluids before they came into the fixative, or in other cases by putting the brains directly into normal salt solution for varying lengths of time. In human material His found the cortical papillæ most marked in material hardened first in formalin and then immersed for several days in Müller's solution. So the same method of fixation was adopted in the experiments, the details of which are as follows:

A. Maceration Followed by Imperfect Fixation.

A1. Maceration in own fluids (11, 12, and 14 cm. pigs).
Embryos left exposed to air, 18 hours.
Embryos placed in formalin, 10%, 48 hours.
Embryos placed in Müller's solution, 4 days.
Washed, brought into alcohol, and then the brain coverings were removed and the brain imbedded and cut in paraffin.

A2. Maceration in normal salt solution (12 cm. pigs).
Embryos placed in salt solution, 17 and 48 hours.
Embryos placed in formalin, 10%, 48 hours.
Embryos placed in Müller's solution, 4 days.
Washed, brought into alcohol, and brain removed as above and the brain then sectioned in celloidin and paraffin.

In these specimens, in which the brain coverings were left intact throughout the period of fixation, no cortical papillæ were found. Embryos of different sizes were tried (A1) for the purpose of covering the whole period favorable to the formation of the papillæ. The poor preservation of the tissues manifested itself by a varying degree of fragmentation of the sections, particularly of the deeper parts. The sections presented a shredded appearance which varied from minute forking clefts between small clumps of cells and between fiber bundles, up to large irregular cracks splitting the different layers of the brain wall. This condition was found both in material that was cut in paraffin and in that cut in celloidin, but it was more marked in specimens that had been macerated in salt solution 17 hours, and still more marked in those macerated 48 hours. Otherwise the general topography of the sections and the arrangement of the layers was fairly well preserved. The minute clefts between the cells of the pyramidal layer gave a slightly ragged appearance to the surface

of that layer, but it was nothing that approched the fungiform arrangement seen in the Retzius papillæ. As can be seen in normal specimens at this time, the pyramidal layer is split by a line of scanty nuclei into a

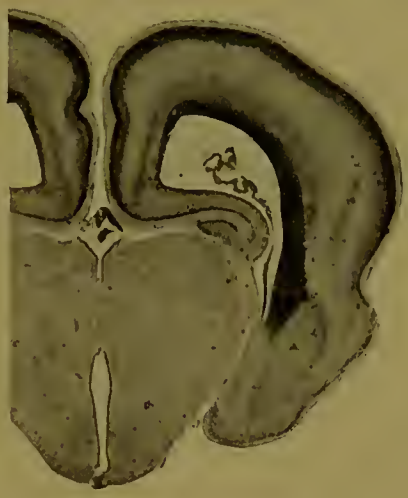

Fig. 3. Section from a well-preserved brain of a pig embryo. 11.5 cm. long. This section shows that normally, in the pig brain of this age, the pyramidal zone presents a uniformly smooth outer surface. This brain, while still warm, was preserved in a chrome-acetic mixture.

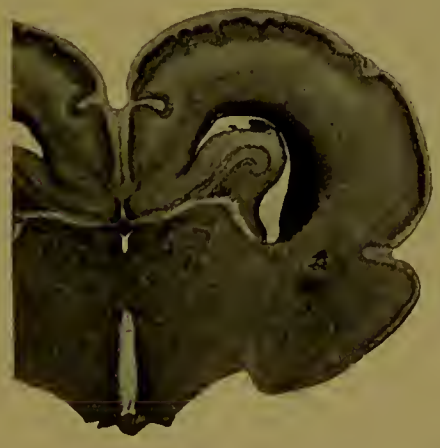

Fig. 4. Section from a macerated brain of a pig embryo, 11.5 cm. long. The brain was kept in normal salt solution 48 hours and then prescrved in formalin followed by Müller's solution. The section shows distinct fungiform clumping of the cortical cells and characteristic Retzius papillæ. The same specimen is shown under higher power in Fig. 6.

more superficial thicker subdivision, the pyramidal cells proper, and a deeper subdivision which is to form the layer of polygonal cells. This stratification was preserved in the experimental material. Another feature of importance was the absence of the so-called transitory fissures.

B. Maceration Followed by Good Fixation.

B1. Maceration in normal salt solution (11.5 cm. pigs)

(a) Fresh brain placed in salt solution, 28 hours.
Hardened in chrome-acetic solution, 48 hours.
Washed, dehydrated and sectioned in celloidin.

(b) (Figs. 4 and 6) Fresh brain placed in salt solution, 48 hours.
Hardened in formalin 10%, 24 hours.
Secondary fixation in Müller's solution, 4 days.
Washed, dehydrated and sectioned in celloidin.

B2. Maceration in its own fluids (11.5 cm. pig) see Fig. 5.
Embryo left exposed to air, 48 hours.
Brain removed and kept in formalin, 48 hours.
Secondary fixation in Müller's solution, 4 days.
Washed, dehydrated and sectioned in celloidin.

The sections of the specimens macerated in salt solution (B1, a and b) show fairly good preservation of the deeper lying parts, there is almost no shredding of the tissue like that seen in the specimens in which the penetration of the fixing fluids was hindered by the brain coverings. The pyramidal layer of the cortex, however, is found to be thrown into irregular folds, accompanied by a fungiform clumping of its constituent cells. This appearance is present in both specimens, but is more marked in the specimen (*b*) macerated 48 hours. A section of this was photographed and is reproduced in Fig. 6. The resemblance is close to the description given by His and Retzius of the cortical papillæ in the human embryo. It has the same smooth-surfaced superficial layer, which dips down between the papillæ of the subjacent pyramidal layer. In some places these incisures cut off small irregular islands of pyramidal cells. The inner surface of the pyramidal layer does not have these sharp notches, but runs across the section in an irregular wavy ill-defined line. In addition to the fungiform clumping of the cortical cells, in some of the sections the so-called transitory fissures are found. These dip sharply inward and invade in some cases more than one-third of the thickness of the brain wall. In the formation of these, the superficial layer is partially folded in with a corresponding cleft on the surface of the brain, which is not the case with the cortical papillæ. It may be noted that the artificial character of transitory fissures has been well established by Hochstetter, 98, and Mall, 03, the latter having examined over fifty embryos and found that according to the effect of various dissociating influences he could obtain macera-

tion in all stages, from simple folding of the brain wall up to conversion of the entire central nervous system into a pulpy mass. Evidently cortical papillæ and transitory fissures, though differing in character, have a similar etiology; as in the above experiment we have both, artificially produced in the same brain under known conditions. The interesting fact should be noted that though the two formations may occur in the same brain, and may closely adjoin each other, yet they do not occur together at the same place; that is to say, one does not find a fungiform grouping

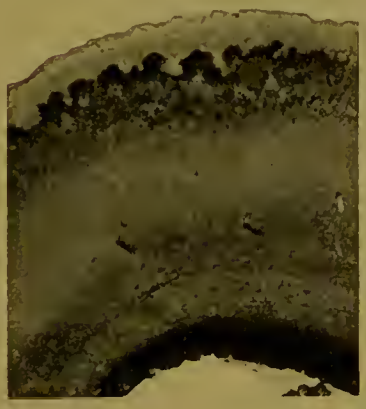

Fig. 5.

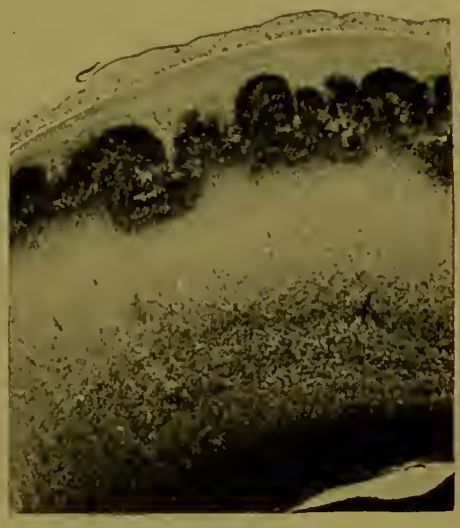

Fig. 6.

Fig. 5. Section of the brain of a pig embryo, 11.5 cm. long. The embryo was left exposed to the air, and the brain allowed to macerate in its own fluid 48 hours. The brain was then removed and preserved in formalin followed by Müller's solution. In this section the fungiform clumping involves only the outer part of the pyramidal zone, and in this respect closely resembles the condition seen in Fig. 1.

Fig. 6. Section of same specimen shown in Fig. 4. The maceration here is more advanced than that seen in Fig. 5. The fungiform clumping involves the whole thickness of the pyramidal zone, both the inner and outer surfaces of which are thrown into coarse irregular folds.

of the cells that lie in the cleft of a transitory fissure. Either process seems sufficient to satisfy the space demand.

Sections from the specimen macerated in its own fluids (B2), see Fig. 5, differ from those macerated directly in salt solution in that the fungiform arrangement of the cortical cells involves only the more superficial part of the pyramidal layer. Instead of foldings of the whole layer, such as is seen in Fig. 6, we have here only a granulated or fungiform surface; and this duplicates almost exactly the condition found in the embryo P1

of His, which he pictures in Figs. 75, 77, 98 and 99. It shows that it is possible by different methods of maceration to produce experimentally typical cortical papillæ in brains where they are not normally present.

Conclusion.

The comparison of Figs. 1 and 2, one with, and one without cortical papillæ, suggests the probability of the abnormal character of the papillæ. One could still perhaps raise the objection that they may be normal, but very transitory, and that the two sections do not quite represent the same stage of development, so that in Fig. 2 the papillæ have either already disappeared or have not yet developed. This objection, however, can no longer be considered in face of the fact that in pigs, where one is able to secure specimens in exactly the same stage of development, it is possible, as has been shown above, to produce the papillæ by means of maceration, and furthermore to control their size and character by varying the degree and method of maceration.

From the experience derived from the above experiments, as regards conditions which predispose to artificial fissures of the cortex and deformities of its constituent cell layers, it becomes evident that embryo brains, which are intended for general morphological study, should, up until the time of completion of the principal fissures, be hardened *in situ* without disturbing the brain coverings. If the brain of a human embryo fresh from the uterus is uncovered or completely removed, and then immediately immersed in formalin or other fixative, it will not necessarily be free from abnormal fissures, etc. The framework of the brain wall up to that time is by no means firm, and it must be also remembered that it may already have been softened by maceration in the uterus. Thus in such a case, and much more so in the embryos that do not reach the hardening fluid so promptly, it is essential that the brain coverings should be left intact, that they may serve as a support to the brain during the process of fixation. Imperfect penetration of the preserving fluid is to be obviated by injecting it through the blood-vascular system.

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